GPT (ALT)

NADH. Kinetic UV. IFCC rec.

Quantitative determination of alanine aminotransferase GPT (ALT)

IVD

PACKAGING

Ref: 101-0255	Cont.: 10 x 10 mL
Ref: 101-0524	Cont.: 4 x 50 mL
Ref: 101-0228	Cont.: 8 x 100 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

The ALT is a cellular enzyme, found in highest concentration in liver and kidney. High levels are observed in hepatic disease like hepatitis, diseases of muscles and traumatisms, its better application is in the diagnosis of the diseases of the liver.
When they are used in conjunction with AST aid in the diagnosis of infarcts in the myocardium, since the value of the ALT stays within the normal limits in the presence of elevated levels of AST[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Alanine aminotranferase (ALT) or Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and piruvate.
The piruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{Piruvate}$$

$$\text{Piruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample[1].

REAGENTS

R 1 Buffer	TRIS pH 7.8	100 mmol/L
	L-Alanine	500 mmol/L
R 2 Substrate	NADH	0.18 mmol/L
	Lactate dehydrogenase (LDH)	1200 U/L
	α -Ketoglutarate	15 mmol/L

PREPARATION

Working reagent (WR):
Ref: 101-0326: Dissolve one tablet of R 2 Substrate with one vial of R1 Buffer.
Ref: 101-0255, 101-0524, 101-0523, 101-0228: Dissolve the contents of R 2 Substrate in the corresponding volume of R 1.
Cap and mix gently to dissolve contents.
Stability: 21 days at 2-8º C or 72 hours at room temperature (15-25º C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.
Do not use the tablets if appears broken.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.00.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25º C, 30° C or 37° C (± 0.1º C).
- Matched cuvettes 1.0 cm light path.
- **General laboratory equipment.**
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SAMPLES

Serum or plasma[1]: Stability 7 days at 2-8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . .25º C / 30º C / 37º C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

WR (mL)	1.0
Sample (µL)	100

4. Mix, incubate for 1 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1-minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

ΔA/min x 1750 = U/L of ALT

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
25º C	1.00	1.32	1.82
30º C	0.76	1.00	1.39
37º C	0.55	0.72	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures:
Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253).

If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[4,5]

	25º C	30º C	37º C
Men	up to 22 U/L	29 U/L	40 U/L
Women	up to 18 U/L	22 U/L	32 U/L

Normal newborns have been reported to show a reference range of up to double the adult, attributed to the neonate's hepatocytes. These values decline to adult levels by approximately 3 months of age.
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.000 U/L to linearity limit of 400 U/L. If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	42	112	41	111
SD	0.47	0.96	0.79	2.21
CV (%)	1.12	0.85	1.90	1.98

Sensitivity: 1 U/L = 0.000503 ΔA / min.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.9869.
Regression equation: y= 1.0589x – 0.6075.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Anticoagulants currently in use like heparin, EDTA, oxalate and fluoride do not affect the results. Hemolysis interferes with the assay[1].
A list of drugs and other interfering substances with ALT determination has been reported[2,3].

BIBLIOGRAPHY

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CHRONOLAB SYSTEMS, S.L., Travessia Prat de la Riba 34 B, 08849 Sant Climent de Llobregat, Barcelona, Spain
Tel. +34 617722466, www.chronolab.com, e-mail: info@chronolab.com